EXPERIMENTAL INSECT TRANSMISSION OF ANTHRAX.

By M. BRUIN MITZMAIN, Veterinary Entomologist, Government of the Philippines.

Only a very few clearly defined instances have been recorded of any experimental evidence demonstrating the rôle of suctorial insects in the dissemination of anthrax. It was with this in view that the writer investigated the purely entomological aspects of the anthrax problem. The question of contamination through skin abrasions produced by insects other than the blood-sucking forms is not at present considered.

This preliminary note is the culmination of a great number of experiments attempted. Only the three positive ones are cited in detail. The difficulty presented is essentially in that the problem of anthrax dissemination is not significant for the suctorial insect until the peripheral circulation becomes invaded with tremendous numbers of the anthrax bacterium. In the first trials the blood donors were employed 24 hours to 3 days prior to their death, and although characteristic cultures were obtained from blood drawn from the ear upon these occasions, no transfer of infection through fly biting resulted in the many experiments attempted.

Only negative results were obtained when it was aimed to demonstrate the possibility of biting fly transmission with animals recently dead of the disease. Both *Stomoxys calcitrans* and *Tabanus striatus* were employed in six experiments in the direct method. The primary host in these instances was used 10 minutes after death. A pure culture of anthrax was obtained during the experiments from the skin in the mucous layer of the region where the flies were applied.

The experiments resulting in the transmission of the anthrax organism were tried with an artificially infected guinea pig, which died of the disease upon the third day. The flies were applied two and one-half hours to a few minutes before the death of the blood donor. Guinea pigs were used to receive the infective bites of *Stomoxys calcitrans* and *Tabanus striatus*. Each animal was placed in a gauze sack strapped to an individual board and the flies were induced to feed when applied from separate test tubes. *Stomoxys calcitrans* was used as the porter in two experiments and *Tabanus striatus* in a single experiment. With both species the infection was successfully transferred by the direct method in which the flies were interrupted while feeding on the sick animal. The stable flies were transferred to the healthy animal in one trial with only a few seconds' interval after biting the infected host, and in the other instance an interval of 10 minutes elapsed between the feedings. A total of 20 flies were used in the first experiment and 30 flies in the second trial.

The exposed animals died in both cases during the evening of the third day. Typical pictures of anthrax infection were presented at the necropsy of the two animals. In addition a substantial gelatinous and hemorrhagic œdema was observed in the subcutaneous region of the area upon which the flies were applied in biting. The spleens of both animals yielded characteristic square-ended rods, which showed typical picture when tested with McFadyean's differential stain. Pure cultures were obtained from the spleens of the dead animals. The growth on agar resembled that of the initial pure culture, and spores produced on a potato medium stained quite typically. The agar cultures when injected reproduced the disease with fatal results in guinea pigs used in later experiments.

Similar results were obtained in all essentials when horseflies were employed to transfer the disease from the sick to a healthy guinea pig. Three flies were used to carry the infection, with only a few seconds' interval from infected to healthy host. The latter died on the fourth day after the flies were applied. The autopsy was made a few minutes after death. As in the other instances, there was no rigor mortis. The site of fly biting was not much involved; only a slight gelatinous hemorrhagic œdema. The subcutaneous injection also was slight. The spleen was greatly enlarged and extremely friable. Stained smears from this organ showed characteristic square-ended rods in great numbers. McFadyean's differential test was very distinct. Numerous nonmotile rods were seen in a hanging drop taken from the heart's blood.

Vigorous growth characteristic of the anthrax bacterium was obtained on agar, and later the disease was reproduced in a horse from a saline suspension of the agar culture.

Typical organisms were seen in the feces of horseflies at various intervals up to 48 hours from the time the infected animal was bitten. The accumulated deposits of 3 tabanids, 2 to 3 days after the infective bites, were injected in a saline suspension into a healthy guinea pig, which died of typical anthrax 4 days later. An agar culture from the spleen of this animal showed typical growth, and a microscopical verification of the character of the organism was obtained.

The feces of the stable fly were likewise found to be infected up to 24 hours after obtaining blood from a sick animal. A nearly pure culture of anthrax was obtained from the droppings of 2 flies fed 24 hours previously on infected material. A mixed culture showing numerous typical rods was obtained from the gut contents of 3 stable flies which were killed 24 hours after biting a sick animal.

A series of experiments is at present being conducted with anthrax in cattle and horses. Guinea pigs or other rodents will in every instance be employed as blood donors, as experience has shown that

it is difficult in large animals to time the probable invasion of the peripheral circulation by the anthrax organisms so as to render insect transmission practicable. An attempt will be made to determine the limits of infection in flies acting as carriers of contaminative material.

EDITOR'S NOTE.—The following message from Manila was received December 31, 1913:

"Anthrax transmission experiments verified *Stomoxys calcitrans* 20 minutes interval harbors bacilli in feces 14 to 17 days inclusive in the stomach 19 days cultures positive *Tabanus striatus* biting direct bacilli in feces 10 days.

"MITZMAIN."